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14. ABSTRACT: We hypothesized that a subset of men who develop prostate cancer (PCa) do so as a result of an inherited chromosomal deletion or amplification, affecting the function of one or more critical prostate cancer susceptibility genes. These chromosomal abnormalities, and the affected gene(s) can be identified using the novel technique, ROMA. Together with our collaborators at Cold Spring Harbor, we performed ROMA analysis of 90 PCa patients, each with a strong family history of PCa (having at least two affected first degree relatives). The majority of these men had either early onset PCa (diagnosis before age 60) or evidence of advanced disease (non-organ confined disease). Over 1100 copy number polymorphism (CNPs), counting many recurring CNPs were observed in these 90 patients, although we were not able to verify many of these CNPs using quantitative PCR. Additionally, we were able to rule out a causal role for a number of CNPs based on lack of cosegregation with disease in families in which multiple affected members have been subjected to ROMA analysis. From assaying a CNP on chromosome 2 in a large population of PCa cases and controls, we were able to show a significant association of this CNP with risk of PCa diagnosis. Currently we are examining other common and rare CNPs for evidence of association with PCa risk.

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INTRODUCTION

While the reasons why prostate cancers occur are incompletely understood, the most well characterized risk factors for prostate cancer are age, race, and family history. Twin studies suggest a substantial portion of familial aggregation of prostate cancer is due to a genetic influence. Segregation analyses provide strong evidence that that one or more major prostate cancer susceptibility gene(s) may be responsible for this genetic influence. However, despite years of extensive effort by multiple research groups world wide, a major gene which is consistently and reproducibly associated with prostate cancer risk has not been identified. In this proposal, we hypothesized that a subset of men who develop prostate cancer do so as a result of an inherited chromosomal deletion or amplification, affecting the function of one or more critical prostate cancer susceptibility genes. These chromosomal abnormalities, and the affected gene(s) can be identified using the novel technique, ROMA. In this respect ROMA provides a fundamental, systematic basis for the identification of prostate cancer susceptibility genes. As such, these efforts may afford significant insight into the basic genetic mechanisms of prostate carcinogenesis. If new ways to prevent and effectively treat prostate cancer are to be other than empirically based, we are in urgent need of additional molecular mechanistic information regarding who is likely to develop prostate cancer and why. Identification of the genes involved in prostate carcinogenesis is a prerequisite condition for answering these questions.

BODY: We have completed Tasks 1 and 2 as described in our SOW.

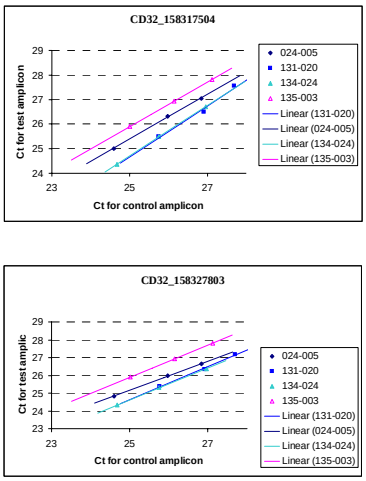
Together with our collaborators at Cold Spring Harbor (CSH), we have now performed ROMA analysis of 90 prostate cancer patients, each with a strong family history of prostate cancer (having at least two affected first degree relatives). The majority of these men had either early onset prostate cancer (diagnosis before age 60) or evidence of advanced disease (non-organ confined disease). Over 1100 CNPs, counting many recurring CNPs have been observed in these 90 patients including 33 novel CNPs which have never (or rarely) been seen in control samples (see Table I for examples). Of these 33 novel CNPs, we can rule out a causal role for at least 5 based on lack of cosegregation with disease in families in which multiple affected members have been subjected to ROMA analysis. An additional 15 novel CNPs do not affect the coding sequences of any known genes. An example of a relatively rare CNP, affecting gene copy number of the CD16 and CD32 immunoglobulin receptors has been confirmed by Q-PCR (shown below in Fig 1).

Table 1 — Examples of CNPs detected by ROMA in HPC probands

Candidate regions: # of probes	Chrom. Band	genes affected	Chrom coordinates Start Pos	Size (bp)	N= 92 HPC set frequencies Up	Down
7	1p36.21	PRAME	12.67Mb	308kb	32	0
9	1q23.3	CD32, CD16	158.2Mb	268kb	7	1
6	4p16.3	BC043151(Zinc finger protein)	4.28Mb	79kb	7	1
4	5q31.3	PCDHA1-13	140.1Mb	16kb	0	15
2	6p21.32	FKBP5, SRPK1	35.7Mb	135kb	0	35
3	7q35	OR2A5,OR6B1	143.0Mb	76kb	1	7
2	13q32.3	EBI2, GPR18, PHGDHL1	98.65Mb	251kb	0	6

Figure 1

QPCR confirms copy number variation at CD32 (FCGR2B)

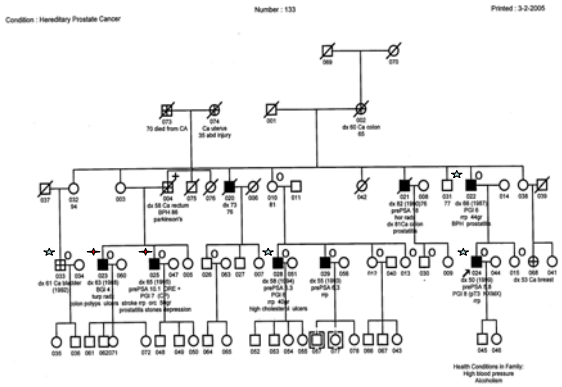


-Both increase and decrease in copy number observed

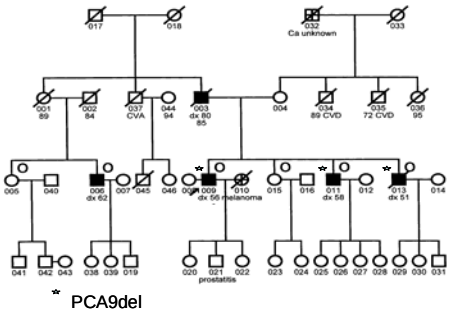
024-005 wt
131-020 increase
134-024 increase
135-003 decrease

Figure 2

Protocadherin Alpha gene deletions in HPC families



☆ PCA9del
+ MSR1 R293X



☆ PCA9del

- PCA9del
- wt

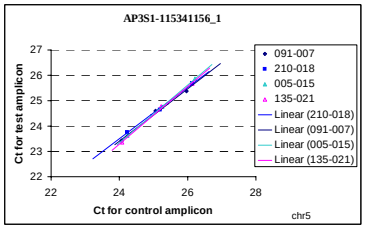
het wt del het wt

No difference in frequency of PCA9del between HPC probands and controls

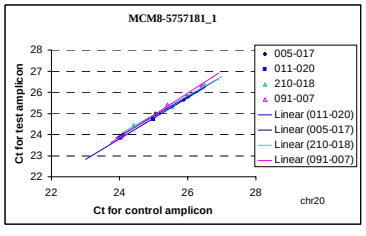
A recurrent germline deletion detected by ROMA and confirmed by PCR which deletes the entire coding for protocadherin A9 on chromosome 5 is shown in Figure 2. We genotyped several of our HPC families for this CNP as well as examined a case-control population. Whereas there was some evidence of disease cosegregation with the deletion, there was no difference in the frequency of the deletion in PCa cases and controls.

Unfortunately we have realized that a substantial fraction of the CNPs detected by ROMA can not be confirmed by QPCR (see Figure 3 for examples). As a complementary approach we have spent considerable effort to examine the ability to detect CNPs using the Affymetrix SNP chip platform (Liu et al 2007). Indeed, both the Affy 100K and 500K SNP chip can detect CNPs that are confirmable by QPCR (see Fig 4-6).

Figure 3



Lack of confirmation by QPCR of deletions detected by ROMA



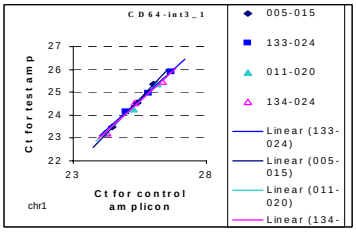


Figure 4

Germline CNPs in HPC proband 005-015
- analysis on Affy 100k SNP chip

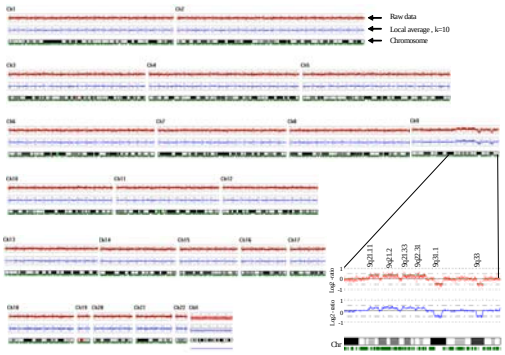
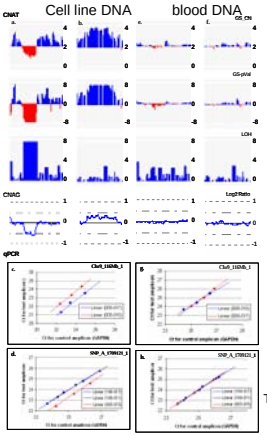


Figure 5

Germline CNPs in LCL and blood DNA (005-015)

- copy number changes acquired in vitro?



Deletion of TLR4 in LCL

no deletion of TLR4 in blood

In the process of carrying out these analyses, we realized that lymphoblastoid cell lines derived from some of our patients may harbor alterations that are not detectable in the blood DNA from the same individual (see Figure 5), suggesting that cell lines may acquire alteration during their preparation or during their time in culture.

Figure 6

Chr 3 CNV detected on Affy 500k Chip

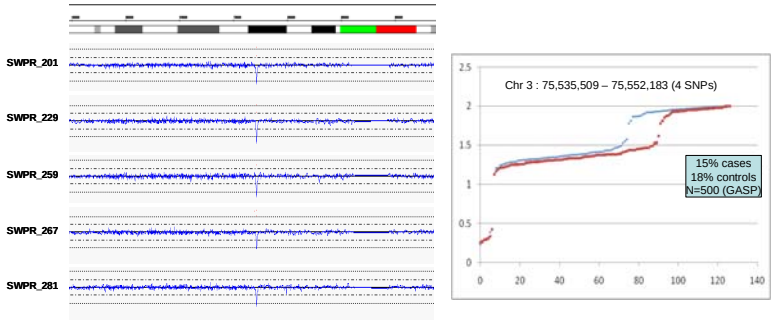


Fig 8. DNA copy number at chromosome 3 among subjects is estimated using the computer program CNAG. A deletion of ~80 kb at 75,535,509-75,621,533 Mb is clearly seen in 5 subjects.

Figure 6 shows a CNP on chromosome 3 detected in 5 different individuals. In initial survey of 500 PCa cases and controls, this CNP was less common in cases (15%) than it was in controls (18%). Testing this CNP in an additional 500 cases and controls showed no difference in frequency however.

We are now characterizing a CNP identified on chromosome 2p using the Affymetrix platform which does show evidence of a reproducible difference in frequency between cases and controls, and may represent one of the first CNPs to be associated with prostate cancer (see data below). Unfortunately, this CNP is not in a gene so its functional significance is unclear at present.

(N = 2,072) PCa cases: 160/1102 = 14.52% Controls: 86/970 = 8.86% $P = 7.4 \times 10^{-5}$

KEY RESEARCH ACCOMPLISHMENTS:

- We have detected many copy number alterations in the germline of HPC cases
- Both ROMA and Affymetrix SNP panels can detect germline CNPs
  - Need to Confirm (qPCR, FISH) to rule out false positives
  - Lymphoblastoid cell lines may harbor alterations not seen in blood DNA
    - Need to confirm in blood
  - Increased resolution provides more information (and more noise!)
  - cross platform validation is an effective and efficient way to identify true CNPs
- identification of a CNV associated with PCa risk, on chromosome 2

REPORTABLE OUTCOMES

1) published manuscript: Liu W, Chang B, Li T, Dimitrov L, Kim S, Kim JW, Turner AR, Meyers DA, Trent JM, Zheng SL, Isaacs WB, Xu J. Germline copy number polymorphisms involving larger than 100 kb are uncommon in normal subjects. Prostate. 2007 Feb 15;67(3):227-33.
2) formation of HPC CNP database

CONCLUSIONS

We proposed to take advantage of the development of the ROMA technique and the existence of the well characterized prostate cancer study populations collected at Hopkins to identify and characterize germline DNA copy number changes in prostate cancer patients. Initially, we have focused on a subset of men who have aggressive prostate cancer at a young age, as we suspect that these men are most likely to harbor rare chromosomal abnormalities responsible for this phenotype. We have identified many common and rare CNPs in the germline of men with prostate cancer. There have been some technical limitations to overcome. We have evaluated the significance of these germline alterations with respect to their possible causal association with prostate cancer, and with one possible exception, have not found reproducible evidence of such an association. Identification of germline genomic deletions or amplifications reproducibly associated with prostate cancer will provide an important basis for understanding the molecular genetics of this common disease.